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Long-Term Effects of Zinc Exposures on Brook Trout (*Salvelinus fontinalis*)

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Abstract

Exposure of three generations of brook trout (*Salvelinus fontinalis*) to zinc concentrations ranging from 2.6 to 534 μg/liter produced no significant harmful effects. During a separate exposure of embryos and larvae, 1,368 μg Zn/liter significantly reduced ($P = 0.05$) both embryo and 12-week larval survival. An additional partial chronic exposure also resulted in significantly reduced ($P = 0.05$) egg chorion strength and embryo survival at 1,360 μg Zn/liter. The maximum acceptable toxicant concentration (MATC) for brook trout exposed to zinc in Lake Superior water (hardness = 45.4 mg/liter as $CaCO_3$; pH = 7.0–7.7) lies between 534 and 1,360 μg Zn/liter. The 96-hour LC50 (median lethal) concentration for brook trout was 2,000 μg Zn/liter; thus the application factor (MATC/96-hour LC50) lies between 0.267 and 0.680. Brook trout gill, liver, kidney, and opercular bone tissues accumulated the greatest amounts of zinc. Edible muscle tissue did not accumulate zinc. Zinc loss from gill and liver from first-generation trout transferred to control water for 12 weeks was 55% and 59%, respectively, based on the total micrograms of zinc per whole tissue. Zinc in kidney tissue (based on the total micrograms of zinc per whole tissue) increased 192%, however, during the 12 weeks in control water.

Zinc is a common trace metal essential in minute quantities for mammals and fish (Vladimirov 1969; Frieden 1972), but larger amounts are known to be toxic to fish and other aquatic organisms. Because zinc is a prevalent component of industrial and mining effluents, which are often discharged into aquatic environments, it is important that its toxicity to aquatic organisms be known.

Several life-cycle tests with fish exposed to zinc have been reported in the literature. Brungs (1969) and Eaton (1973) reported that zinc affected the reproduction of fathead minnows (*Pimephales promelas*) in hard water, and they concluded that reduced egg production was the most sensitive indicator of toxicity. Spehar (1976) also noted an apparent reduction in the number of eggs produced by flagfish (*Jordanella floridae*) exposed to zinc. However, Benoit and Holcombe (1978) showed that egg adhesiveness and fragility (measured by the pressure required to rupture the chorion) were responsible for low egg production and not direct effects of the metal on parental fish. Sinley et al. (1974) and Chapman (1978) do not include information on natural spawning in their reports of long-term tests with zinc and salmonids. Since brook trout (*Salvelinus fontinalis*) had spawned successfully during previous chronic metal studies (McKim and Benoit 1971, 1974; Benoit 1976; Holcombe et al. 1976; Benoit et al. 1976; McKim et al. 1976), they were considered an excellent test fish for further evaluation of the effects of zinc on reproduction.

The present study was designed to determine the effects of zinc on survival, growth, and reproduction of brook trout and to ascertain the uptake, distribution and elimination of zinc in the tissues of trout. Other objectives were to determine (1) the most sensitive life stage of brook trout to zinc; (2) the effect of acclimation on toxicity, if any; and (3) the maximum acceptable toxicant concentration (MATC) to allow the calculation of an application factor (AF) for zinc (Mount and Stephan 1967) that could be evaluated and compared with application factors calculated for other species.

Methods

Exposure System

Two proportional diluters (Mount and Brungs 1967) in two separate identical exposure systems each delivered 2 liters of test water per cycle to separate flow-splitting chambers (Benoit and Puglisi 1973). These chambers divided each of five zinc concentrations and one control four ways into duplicate adult spawning

tanks and duplicate larval growth tanks. Flow rates in the adult spawning tanks and larval growth tanks averaged 620 and 92 ml/minute, respectively. Replacement time for 90% of the test water in the spawning and larval growth tanks of both systems was 4.5 hours (Sprague 1969). System design, tank dimensions, water depth, aeration, tank-cleaning procedures, tank surroundings, and lighting were described by Benoit et al. (1976) and Holcombe et al. (1976).

Water Characteristics

Lake Superior water, used for all exposures, was passed through an ultraviolet sterilizer to kill possible pathogens and then was heated to the proper test temperature by a hot-water heat-exchange system. Seasonally adjusted temperatures ranged from 5 to 15 C throughout the juvenile-adult exposures. Temperatures were maintained at 9 ± 1 C during all spawning, embryo-incubation, and 12-week larval-juvenile growth studies. Total hardness, alkalinity, acidity, and pH were determined for all tanks, alternately, one tank per week; dissolved oxygen was measured in a different tank twice a week. The means and ranges for total hardness, alkalinity, and acidity were 45.4 (43.0–47.5), 41.8 (40.0–44.0), and 2.9 (1.3–6.3) mg/liter as $CaCO_3$, respectively, for the chronic exposures, and 45.9 (45.0–48.0), 41.3 (39.0–43.0), and 2.3 (1.0–7.3) mg/liter as $CaCO_3$, respectively, for the separate embryo-larval exposure. The pH ranged from 7.0 to 7.7 for the chronic tests and from 7.2 to 7.9 in the embryo-larval exposure. The mean concentration of dissolved oxygen was 9.8 mg/liter (range 6.4–12.1) in the chronic exposures and 11.6 mg/liter (range 10.9–12.2) in the embryo-larval test. Methods described by the American Public Health Association et al. (1971) were used to make chemical measurements.

Toxicant Solution

Zinc sulfate (granular, $ZnSO_4 \cdot 7H_2O$, reagent grade) was dissolved in distilled water and introduced to the diluters by a chemical-metering device (Mount and Warner 1965). Zinc concentrations of duplicate tanks and for combined duplicate tanks for first-, second-, and third-generation studies (Table 1 and Table 2, respectively) and for the additional embryo-larval test (Table 3) were assigned to the test tanks by a stratified random procedure.

Water samples (25 ml/day) were taken Monday through Friday from all exposure tanks with fish present, acidified to pH 3.0 and composited over a 2-week period for zinc analyses. This procedure provided 141 analyses for the three-generation exposure and 12 analyses for the embryo-larval study. Zinc concentrations were determined by flame atomic absorption spectrophotometry (Perkin-Elmer 1976) and compared to reference metal samples from the United States Environmental Monitoring and Support Laboratory in Cincinnati. Spiked samples were analyzed to determine percentage recoveries. The mean percentage recovery and 95% confidence interval of spiked samples was 102 ± 0.5% (N = 101). The analyzed values and 95% confidence intervals of the three reference metal samples were 10 ± 0.7, 78 ± 1.3, and 371 ± 2.7 μg Zn/liter (N = 53); the actual values were 10, 75, and 367 μg Zn/liter.

Biological Protocol

The tests in this study included (1) an initial exposure of brook trout from the yearling stage through adult spawning (June 1974–November 1974; first generation); (2) exposure of the offspring of the initial parental group from embryo stage through adult spawning (October 1974–December 1976; second generation); (3) exposure of the offspring of the second parental group from embryo to the early juvenile stage (October 1976–March 1977; third generation) (Fig. 1); (4) a separate exposure of embryos and larvae as they grew to the early juvenile stage (October 1975–March 1976); and (5) a second continuous exposure of fish from yearling through spawning and of their offspring through the early juvenile stage (May 1976–March 1977). Biological methods for these groups will be given separately.

First Generation

Yearling brook trout (about 70 g) obtained from Cedar Bend Trout Farm in Scandia, Minnesota, were held in acclimation tanks for 2 weeks. Twenty-four fish were placed in each of the 12 exposure tanks by stratified random assignment. Fish were then acclimated for 2 additional weeks before the zinc exposure was begun in June 1974. During September 1974, all remaining fish in each tank were separated into groups of males and females. From these groups spawners were chosen randomly so that

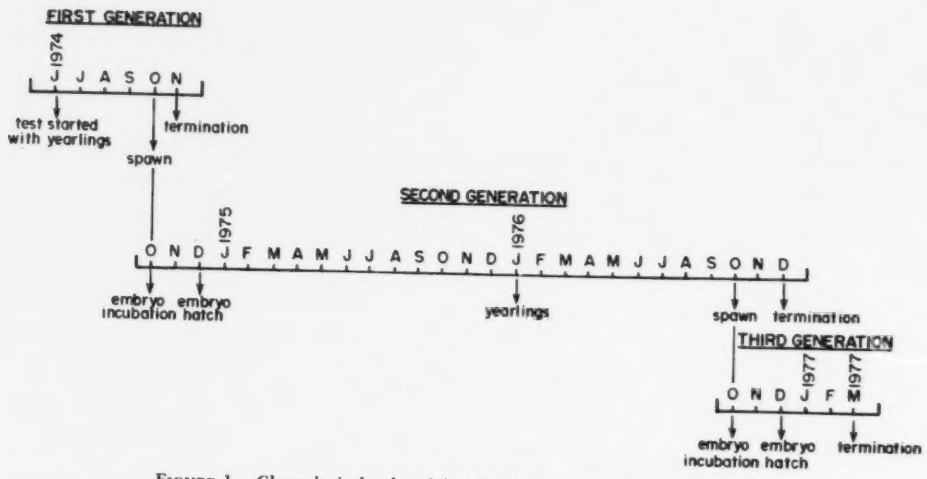

FIGURE 1.—*Chronological order of three brook trout generations exposed to zinc.*

each duplicate tank contained two males and four females. One 28 × 33 cm by 13-cm-deep stainless steel pan containing 5 cm of loose gravel was placed in each tank, and spawning commenced in October 1974. Experimental methods used during spawning and parental termination were described by McKim and Benoit (1971).

After exposure periods of 4, 16, and 24 weeks, subsamples of two to five fish per duplicate were anesthetized with MS-222 (ethyl *m*-aminobenzoate methanesulphonate). Gill, liver, kidney, muscle, spleen, gonad, brain, skin, and opercular bone tissues were dissected, rinsed with distilled water, individually wrapped in cellophane, and frozen for subsequent zinc analysis. After 24 weeks' exposure two fish per duplicate from 2.6 (control) and 534 μg Zn/liter were transferred to control water. After 12 weeks in control water, these fish were anesthetized, and gill, liver, and kidney tissues dissected and analyzed to determine the amount of zinc eliminated from these tissues. Opercular bone tissues were not examined during the elimination phase. All 4-week tissue samples were analyzed individually, whereas the 16-, 24-, and 12-week tissue samples used for determining depuration were pooled in two equal samples and analyzed.

Zinc residues in brook trout tissues, embryos, and larvae were determined by flame atomic absorption spectrophotometry following wet digestion with a 1:1 mixture of nitric and perchloric acids (Leonard 1971). The mean percentage recovery and 95% confidence interval of zinc spiked into tissue samples was 101 ± 4.1% ($N = 20$).

Second Generation

Exposure of this generation through one complete life cycle began with the spawning of first-generation fish (Fig. 1). Methods used during embryo incubation and the larval-juvenile growth studies were the same as those described by McKim and Benoit (1974). Ten freshly spawned eggs and 10 newly hatched larvae per duplicate were preserved in 4% formaldehyde solution for later zinc-residue analysis. Weights were determined on a subsample of 12 fish per concentration 12 weeks after hatching. Juveniles were transferred from the larval growth chambers to the adult spawning tanks 20 weeks after hatching. All fish were randomly thinned in Septermber 1976 to the same sex ratio used for the first-generation fish, and one artificial spawning substrate (Benoit 1974) was placed in each tank. Spawning commenced at the end of September and continued into November. Methods used for spawning and parental termination were the same as those used for first-generation fish. Gill, liver, kidney, spleen, muscle, and gonad tissues were dissected from two to five randomly selected fish from each duplicate at 52 and 93 weeks

after hatch. Tissue samples were pooled to obtain two samples per concentration and analyzed for zinc as previously described for first-generation fish.

Third Generation

Exposure of this generation began with the spawning of the second-generation fish. Methods used for this generation were the same as those described for second-generation embryo-juvenile exposures except that all fish were terminated 12 weeks after hatch.

Egg chorion strength (fragility) of third-generation brook trout was examined because a previous study indicated that zinc affected the chorion of fathead minnows (Benoit and Holcombe 1978). Subsamples of five viable eggs from selected spawnings in zinc concentrations of 1,360, 534, 266, 144, and 2.6 μg/liter were checked for egg fragility 11 days after they were spawned. Individual eggs were placed on a tared scale in a 9-mm glass tube that was inserted into a hard rubber stopper base, a 5-mm piece of capillary tubing was then placed on top of the egg, and gentle pressure was applied to the capillary tube. Egg fragility was measured by the number of grams required to rupture each chorion.

Several eggs stripped and fertilized from extra second-generation control fish were also transferred to the control and 1,360 μg Zn/liter exposures before (within 4 minutes of fertilization) and after (4 hours after fertilization) the eggs had become water-hardened to observe the effects, if any, of this procedure on the chorion strength of 11-day-old embryos.

Separate Embryo-Larval Exposure

During October 1975 stripped brook trout eggs (about 6 hours old) obtained from Cedar Bend Trout Farm in Scandia, Minnesota, were placed in each of 12 tanks for larval growth that comprised a separate diluter system, and in the 534, 266, and 2.6 (control) μg Zn/liter larval tanks in the life-cycle exposure system. This test, conducted with higher zinc concentrations, was included because no definite zinc-related effects had been observed thus far in the life-cycle exposure. Two egg cups containing 50 embryos per cup were placed in each tank. In addition, 500 eggs (about 6 hours old) were incubated in the control larval tanks. At hatch, half of these control larvae were transferred to zinc concentrations, and the other half were transferred during swim-up (4 weeks after hatching) to determine possible acclimation effects. Methods used during embryo incubation and larval-juvenile growth studies were described by McKim and Benoit (1974). All fish were killed 12 weeks after hatch.

Additional Yearling Exposure

An additional group of yearling brook trout was continuously exposed to the lowest zinc concentration tested (1,368 μg/liter) that had significant effects on embryo-larval and early juvenile stages. These 73-week-old second-generation control fish were transferred to water containing 1,368 μg Zn/liter in May 1976 to ascertain if there were effects on adult fish or reproduction, or both, at zinc concentrations that were found harmful to brook trout embryos and larvae and to confirm the effects seen previously on embryos and larvae at this concentration. Methods used during spawning, parental termination, embryo incubation, and 12-week larval exposure were the same as those described for second-generation fish in the full life-cycle test. After 20 weeks' exposure five fish per duplicate were randomly selected and dissected for tissue analyses as was done for first-generation fish.

Trout Diets and Treatment of Disease

All fish were fed measured amounts of a commerical trout food (Glencoe Mills[1], Ewos[2], and Zeigler Bros.[3]) daily. Food was given at rates ranging from 0.5% to 3.4% of group body weights, depending on the average weight of fish and water temperature. Rations of second-generation fish were reduced 5% and 25% below the regular feeding schedule at 52 and 73 weeks, respectively, to provide a maintenance diet that would prevent fish from outgrowing the spawning substrates used in the test.

First-generation trout were treated once prophylactically with 150 mg/liter formalin before spawning, and several second-generation trout were anesthetized and swabbed with a 1% so-

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[1] Glencoe Mills, Incorporated, Glencoe, Minnesota 55336.

[2] Astra Pharmaceutical Company, Worcester, Massachusetts 01606.

[3] Zeigler Bros., Incorporated, Gardners, Pennsylvania 17324.

TABLE 1.—*Growth, survival, and reproduction of first- and second-generation brook trout exposed to various concentrations of zinc. A and B represent duplicate spawning tanks.*

Item	Total zinc[a] (μg/liter)						
	1,366.4[b] ±26.9 A	1,353.0[b] ±38.5 B	534.4 ±8.4 A	532.7 ±8.9 B	267.9 ±4.1 A	264.6 ±4.6 B	147.5 ±2.5 A
First generation							
Average weight of fish before spawning (g)[a]	161.4 ±44.8	164.7 ±43.6	102.0 ±24.3	102.6 ±20.8	126.9 ±30.6	121.6 ±42.0	108.2 ±14.0
Adult survival during spawning (Oct 1974–Nov 1974) (%)	100	100	100	100	100	100	100
Mean spawnings per female	2.3	3.8	1.5	1.5	2.3	2.3	1.5
Mean viable eggs spawned per female	348	244	258	356	413	247	303
Second generation							
Average weight of fish before spawning (g)[a]			183.3 ±33.1	181.3 ±37.7	168.4 ±51.0	202.5 ±32.6	173.3 ±27.4
Adult survival during spawning (Sept 1975–Nov 1976) (%)			100	100	100	100	100
Mean spawnings per female			3.4	2.5	2.3	4.0	2.0
Mean viable eggs spawned per female			291	786	450	907	330

Item	Total zinc[a] (μg/liter)						
						Control	
	139.5 ±2.7 B	69.8 ±1.4 A	69.0 ±1.6 B	39.5 ±1.2 A	39.3 ±1.3 B	2.8 ±0.5 A	2.3 ±0.4 B
First generation							
Average weight of fish before spawning (g)[a]	96.5 ±21.6	121.8 ±31.9	97.7 ±27.9	106.8 ±21.8	99.9 ±23.8	120.6 ±15.7	92.7 ±16.9
Adult survival during spawning (Oct 1974–Nov 1974) (%)	100	100	100	83	100	100	100
Mean spawnings per female	2.0	1.3	2.3	2.3	2.3	2.0	2.0
Mean viable eggs spawned per female	338	211	447	318	646	301	238
Second generation							
Average weight of fish before spawning (g)[a]	173.1 ±48.5	162.8 ±39.7	162.9 ±55.6	148.6 ±39.0	178.9 ±32.1	141.7 ±46.8	176.2 ±55.9
Adult survival during spawning (Sept 1975–Nov 1976) (%)	100	100	100	100	100	100	100
Mean spawnings per female	3.5	3.0	1.8	2.5	2.8	3.1	2.6
Mean viable eggs spawned per female	188	296	235	391	334	154	272

[a] Mean ±95% confidence interval.
[b] Data at this concentration were obtained from a second exposure of yearling brook trout (see text).

lution of zinc-free malachite green for treatment of fin rot.

Data Analysis

Survival, growth, reproduction, and egg-fragility data were subjected to one-way analysis of variance with an F-test of significant ($P = 0.05$) and subsequent analysis with Dunnett's procedure ($P = 0.05$) (Steel and Torrie 1960). Growth and reproduction data were also subjected to a nested analysis of variance with an F-test of significance ($P = 0.05$) (Steel and Torrie 1960) to determine if there were statistical differences between duplicates. The estimated total weight and the ratio (exposed:control) of the estimated total weight of 12-week-old young per female were calculated as described by McKim and Benoit (1974).

Acute Toxicity Tests

Three 96-hour acute zinc toxicity tests were conducted with brook trout in Lake Superior water. Methods used for these tests have been described by Holcombe and Andrew (1978).

TABLE 2.—*Estimated total weight of 12-week-old second- and third-generation brook trout produced per female at various zinc concentrations. Asterisks (*) denote values significantly less than controls (analysis of variance with Dunnett's procedure, P = 0.05).*

Mean total zinc concentration (μg/liter)	Duplicates	A Estimated[a] eggs/female	B Hatchability (%)[b]	C Larval-juvenile survival (%)[c]	D Individual juvenile weight (g)[d]	A × B × C × D Estimated total weight of young per female (g)	Ratio of estimated total weight of young per female (exposed: control)	Mean weight required to rupture egg membrane after 11 days' exposure to zinc (g)[e]
				Second generation				
1,360[f]	A	348	45* (6)	86	0.39 ± 0.05 (42)	52.5	0.72	182* ± 121 (6)
	B	244	82* (3)	98	0.56 ± 0.04 (49)	109.8		
534	A	258	94 (4)	98	0.45 ± 0.18 (6)	107.0	0.96	
	B	356	80* (5)	98	0.39 ± 0.19 (6)	108.9		
266	A	413	99 (8)	94	0.41 ± 0.32 (6)	157.6	1.33	
	B	247	91 (4)	96	0.65 ± 0.24 (6)	140.3		
144	A	303	97 (5)	84	0.30 ± 0.19 (6)	74.1	1.09	
	B	338	96 (8)	94	0.56 ± 0.19 (6)	170.8		
69	A	211	99 (4)	98	0.36 ± 0.15 (6)	73.7	0.96	
	B	447	97 (8)	96	0.34 ± 0.18 (6)	141.5		
39	A	318	100 (6)	96	0.46 ± 0.13 (6)	140.4	1.41	
	B	646	99 (8)	84	0.33 ± 0.13 (6)	177.3		
2.6 (control)	A	301	100 (7)	98	0.42 ± 0.09 (6)	123.9	1.00	
	B	238	98 (5)	94	0.46 ± 0.21 (6)	100.9		
				Third generation				
534	A	276	74 (4)	92	0.58 ± 0.06 (46)	109.0	1.59	971* ± 111 (15)
	B	747	88 (5)	64	0.20 ± 0.05 (30)	84.1		
266	A	428	97 (5)	76	0.37 ± 0.08 (38)	116.7	2.45	993* ± 179 (7)
	B	861	86 (4)	82	0.30 ± 0.06 (41)	182.2		
144	A	314	99 (5)	98	0.46 ± 0.04 (49)	140.1	1.68	1,155 ± 64 (2)
	B	179	92 (5)	96	0.41 ± 0.03 (48)	64.8		
69	A	281	92 (5)	84	0.13 ± 0.03 (41)	28.2	0.60	
	B	223	94 (3)	90	0.24 ± 0.06 (45)	45.3		
39	A	371	98 (5)	86	0.29 ± 0.04 (43)	90.7	1.35	
	B	317	91 (4)	88	0.29 ± 0.05 (44)	73.6		
2.6 (control)	A	146	73 (3)	82	0.25 ± 0.05 (41)	21.8	1.00	1,223 ± 107 (11)
	B	258	81 (4)	92	0.52 ± 0.05 (45)	100.0		

[a] Number of viable embryos produced per spawning female × the probability of spawning (that is, total spawners/total females = 1.0 for the second generation and 0.95 for the third generation).

[b] Numbers of spawnings checked for hatchability are in parentheses.

[c] Fifty fish were exposed in each duplicate.

[d] Mean ± 95% confidence interval. Numbers of fish sampled are in parentheses.

[e] Mean ± 95% confidence interval. Numbers of groups of five eggs examined are in parentheses. Missing entries indicate that fragility was not measured.

[f] Data from this concentration were obtained from a second exposure of yearling brook trout (see text).

Results

First Generation

Neither survival nor growth of first-generation trout were significantly reduced by zinc during either the 24-week exposure or during the 28-week exposures to 1,360 μg Zn/liter (Table 1). Fish exposed to 1,360 μg Zn/liter were much larger than other first-generation fish at the time the tests were initiated, so these obvious size differences were not due to zinc exposure. Spawning occurred in all duplicate exposures, and no significant difference in the mean number of viable eggs produced per female was observed (Table 1).

TABLE 3.—*Percent survival of brook trout transferred to zinc as embryos; at hatch; and 4 weeks after hatch. Asterisks (*) denote values significantly less than controls (analysis of variance, Dunnett's procedure, P = 0.05). Missing entries indicate that no fish were exposed. A and B represent duplicates.*

Item	Total zinc (μg/liter), mean ± 95% confidence interval								
	4,363 ±223.8 A	4,336 ±233.2 B	2,099 ±80.3 A	2,017 ±101.2 B	1,353 ±57.3 A	1,382 ±88.8 B	709 ±60.9 A	724 ±66.6 B	343 ±20.6 A
Embryo (6 hours old) survival to hatch[b]	1.5*	3*	73*	67*	79*	90*	95	97	93
Larvae survival 12 weeks after hatch[c]	0*	0*	58*	70*	68*	74*	88	92	94
12-week survival of larvae transferred to zinc at hatch[d]	4	0	76		92		96		100
8-week survival of larvae transferred to zinc 4 weeks after hatch[d]		0		16		56		96	

Item	Total zinc (μg/liter), mean ± 96% confidence interval								
	364 ±21.7 B	534[a] ±8.4 A	533[a] ±8.9 B	268[a] ±4.1 A	265[a] ±4.6 B	2.8[a] ±0.5 A	2.3[a] ±0.4 B	3.2 ±1.6 A	3.2 ±1.5 B
Embryo (6 hours old) survival to hatch[b]	93	98	91	96	96	98	94	100	95
Larvae survival 12 weeks after hatch[c]	98	96	100	96	98	100	88	92	96
12-week survival of larvae transferred to zinc at hatch[d]								100	100
8-week survival of larvae transferred to zinc 4 weeks after hatch[d]	100	96	100	96	100	88	88		

[a] Embryos and larvae were exposed in larval growth chambers in the life-cycle exposure system.
[b] 100 eggs were exposed per duplicate.
[c] 50 larvae were exposed per duplicate.
[d] 25 larvae were exposed in each test chamber.

Second Generation

Hatchability of second-generation embryos was significantly reduced ($P = 0.05$) in one exposure to 534 μg Zn/liter and in both exposures to 1,360 μg Zn/liter (Table 2). Twelve-week larval survival and growth, however, were not significantly affected by any zinc concentration tested.

The estimated number of eggs per female, percentage hatchability, percentage larval-juvenile survival, and individual juvenile weights were multiplied in succession to yield an estimated total weight of 12-week-old young per female for each exposure concentration. These values were then divided by the estimated total weight of young per female in the controls to yield a ratio which was used to compare estimated total weight of young produced at each zinc concentration to the controls (Table 2). The only large decrease in this ratio below 1.00 resulted from exposures to 1,360 μg/liter, and this decrease was caused mainly by reduced hatchability.

Growth was slightly enhanced in second-generation trout after 73 weeks exposure to 266 and 534 μg Zn/liter. Spawning occurred in all test exposures, and no significant decrease in the mean viable eggs produced per female was observed (Table 1).

Third Generation

Third-generation embryo hatchability was not adversely affected by the zinc concentrations tested (Table 2). Larval-juvenile survival was reduced in one duplicate tank at 534 μg Zn/liter, but this survival value, when combined

with that of the other duplicate, was not enough to be significantly different from the controls. Twelve-week growth data were quite variable, which precluded determining any zinc-related growth effects. Poor spawning, reduced hatchability, and poor growth in one duplicate control tank resulted in a very low estimated total weight of 12-week-old young per female for that tank. This produced a ratio of estimated total weight of young per female in the control that was smaller than the ratios in other exposure concentrations (Table 2).

Third-Generation Egg Fragility

The force required to rupture egg chorions varied inversely with the zinc concentration to which they were exposed (Table 2). Mean chorion strengths were reduced to 94%, 81%, 79%, and 15% of the mean control value after 11 days exposure to 144, 266, 534, and 1,360 μg Zn/liter, respectively. The fragility of control eggs and that of eggs exposed to concentrations of 266 μg Zn/liter and above were significantly different ($P = 0.05$).

Eggs from control fish transferred to 1,360 μg Zn/liter before water hardening ruptured at a mean force of only 116 g, whereas eggs from the same group transferred after water hardening required a mean force of 536 g to rupture the chorion. Fragility for this same group of eggs incubated in control water averaged 1,187 g.

Additional Embryo-Larval Exposure

Survival of brook trout embryos exposed 6 hours after fertilization until hatching was significantly reduced ($P = 0.05$) by zinc concentrations of 1,368 μg Zn/liter and above (Table 3). Survival of 12-week-old acclimated larvae exposed as embryos (6 hours old) was also significantly reduced from controls ($P = 0.05$) at 1,368, 2,058, and 4,350 μg Zn/liter. Concentrations of 2,058 μg Zn/liter and above apparently affected 12-week survival of unacclimated brook trout larvae transferred at hatch, and an 8-week exposure to 1,368 μg/liter and above reduced survival of 12-week-old unacclimated larvae transferred at swim-up (4 weeks after hatch).

The weight of 12-week-old larvae was quite variable among zinc concentrations, but the difference between controls and fish exposed to 2,058 μg Zn/liter was not significant.

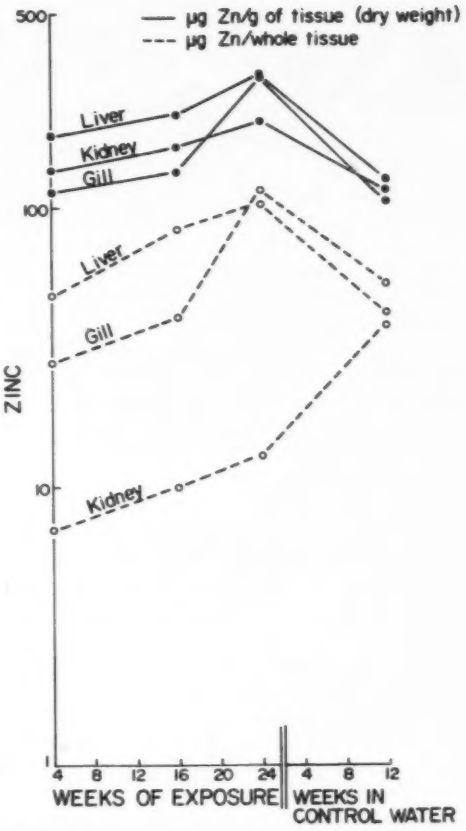

FIGURE 2.—*Zinc residues in tissues of first-generation brook trout exposed to 534 μg Zn/liter, and depuration of residues from tissues of brook trout in control water. Each point represents the mean of 10 samples during the uptake exposure and two samples during the elimination phase.*

Tissue Residues

No significant accumulations of zinc on a microgram-per-gram basis were found in any tissues from first-generation fish after 4 weeks exposure. After 16 weeks exposure to 534 μg Zn/liter, however, and after 24 weeks of exposure at all other zinc concentrations, accumulations of zinc slightly higher than those in controls were present in gill, liver, and kidney tissues. Substantial uptake of zinc residues occurred in gill, liver, and kidney tissues of first-generation trout after 24 weeks exposure to 534 μg Zn/liter (Fig. 2). After 24 weeks expo-

sure to 534 μg Zn/liter both total zinc (μg) present in the gill and the zinc (μg) per gram of gill tissue were approximately three times higher than those in control tissues, and the concentrations in liver and kidney were approximately two times the concentrations in control tissues.

Muscle and brain tissues did not appear to accumulate zinc at any concentration tested. Zinc concentrations in spleen, gonad, and skin from first-generation trout were variable, which made it difficult to observe trends in zinc uptake. However, these tissues did not accumulate zinc to any substantial degree. After 24 weeks exposure, opercular bone had accumulated zinc levels of 309 and 401 μg Zn per gram of tissue at concentrations of 266 and 534 μg Zn/liter, respectively, which were substantially higher amounts than in the controls (149 μg Zn per gram of tissue).

Zinc loss from gill and liver tissue of brook trout exposed to 534 μg Zn/liter for 24 weeks and then transferred to control water for 12 weeks was 55% and 59%, respectively (Fig. 2), but the amount of zinc present in kidney tissue (based on the total micrograms of zinc per whole tissue) increased by 192% during the 12 weeks in control water (Fig. 2).

Gill, liver, kidney, and opercular bone dissected from brook trout exposed to 1,360 μg Zn/liter for 20 weeks contained 4.6, 2.9, 1.6, and 5.4 times more zinc, respectively, than similar tissues from control fish (based on micrograms of zinc per gram of tissue). Spleen, muscle, and gonad tissues did not accumulate any significant residues within 20 weeks.

Second-generation eggs (24 hours old) did not contain significant residues at any concentration. Residues in newly hatched larvae exposed to 2.6, 144, 266, and 534 μg Zn/liter were 6.7, 15.3, 19.3, and 22.6 μg Zn/g tissue, respectively.

Tissues of second-generation trout accumulated less zinc after 93 weeks of exposure than first-generation trout accumulated in 24 weeks, but trends of zinc uptake in second-generation trout were similar to those in first-generation fish. At 52 weeks only gill tissue had accumulated residues higher than control levels, but after 93 weeks the concentrations of zinc in gill, liver, and kidney tissues from fish exposed to 534 μg Zn/liter were 1.8, 1.6, and 1.8 times higher than control concentrations.

Acute Toxicity

The mean of three 96-hour LC50 (median lethal) concentrations determined from acute toxicity tests conducted at 15.0 ± 1 C on juvenile brook trout in soft water was 2,000 μg Zn/liter. Detailed results of these tests are included in an earlier report (Holcombe and Andrew, in press).

MATC and Application Factors

Effects of zinc on egg fragility, embryo survival, and subsequent survival of larval brook trout demonstrated that the maximum acceptable toxicant concentration (MATC) in this water is between 534 and 1,360 μg Zn/liter. The resulting application factor (MATC/96-hour LC50) for brook trout and zinc is between 0.267 and 0.680.

Discussion

Adverse effects occurred primarily during the embryo-larval and early juvenile stages of brook trout development, which indicated that these life stages are the most sensitive to zinc. Zinc did not adversely affect reproduction in brook trout at the concentrations tested, which agrees with results obtained by Benoit and Holcombe (1978) during a fathead minnow life-cycle study with zinc.

Growth was not significantly reduced by zinc during the embryo-larval or adult exposures during this study. These results agree with those from two chronic tests with salmonids. Chapman (1978) determined that the growth of sockeye salmon (*Oncorhynchus nerka*) was slightly stimulated by zinc concentrations as high as 242 μg/liter. Sinley et al. (1974) found that zinc did not significantly affect the growth of rainbow trout (*Salmo gairdneri*) in either hard or soft water at concentrations of 2,200 and 547 μg Zn/liter, respectively. The slight growth increases observed in 73-week-old second-generation trout in the present study are possibly explained by the essential need of small quantities of zinc for metabolism (Frieden 1972; Giesy and Wiener 1977); low concentrations that do not produce toxic effects could stimulate growth. Growth reductions caused by zinc, however, were reported for flagfish (Sephar 1976), fathead minnows (Brungs 1969), guppies (*Lebistes reticulatus*) (Crandell and Goodnight 1962), and the minnow *Phoxinus phoxinus* (Bengtsson 1974).

Zinc exposure caused the chorion of brook trout eggs to become more fragile and more easily ruptured. This effect is important in brook trout because during natural spawning eggs are buried beneath several inches of loose gravel, and weakened eggs would be more likely to rupture by pressure or movement of the gravel than would normal eggs. Although fragility was significantly affected at several zinc concentrations, only 1,360 μg Zn/liter appeared to reduce chorion strength drastically enough to cause possible serious problems during natural spawning in loose gravel. Similar effects of zinc on fragility of fathead minnow chorions in soft water were also well documented by Benoit and Holcombe (1978). Weakened chorions developed in brook trout eggs transferred to water containing zinc both before and after water hardening, but the weakening effect was more pronounced for eggs transferred before water hardening occurred. This result indicates that zinc interferes with either the water-hardening process or the tissues involved with this process. Hurley and Fisher (1965) found that variation of the calcium ion concentration of the water varied the degree of hardening finally attained in brook trout eggs. Since both zinc and calcium ions have the same positive charge, zinc may compete with calcium during the hardening process. Further study would be needed to determine the exact physiological process by which zinc reduces chorion strength and why this effect is lessened after the eggs are water-hardened.

The stage at which zinc exposure was initiated did not significantly affect the survival of 12-week-old brook trout. Larvae from exposed embryos (6 hours old) were slightly less sensitive than larvae from the same group of eggs when the exposure began 4 weeks after hatch (swim-up stage), although the zinc concentration causing an effect was the same. Larvae transferred to zinc shortly after hatching appeared to be even more resistant than either the group exposed as embryos or the group exposed 4 weeks after hatch. Apparently brook trout may acclimate somewhat during the embryo and early larval stage. Results from the present study are similar to those obtained by Benoit and Holcombe (in press). They observed that the 8-week survival of fathead minnows exposed to zinc as embryos was slightly higher than that of fathead minnows exposed as newly hatched larvae. Acclimation effects of zinc on brook trout observed in this study were not nearly so great as those observed by Spehar (1976) for flagfish and Sinley et al. (1974) for rainbow trout. These authors observed differences of nearly three and four times, respectively, between fish exposed as embryos and those exposed as larvae. These previously mentioned acclimation effects could possibly be explained by the formation or activation of enzymes or enzyme systems that help eliminate zinc or internally control tissue concentrations (Giesy and Wiener 1977).

The greatest accumulation of zinc in first- and second-generation brook trout occurred in the gill, liver, kidney, and opercular bone tissues. Other investigators found that gill, liver, and kidney tissues of brown bullheads (*Ictalurus nebulosus;* Joyner 1961) and threespine sticklebacks (*Gasterosteus aculeatus;* Matthiessen and Brafield 1977) also accumulate zinc readily. In the present study, tissues dissected from fish that had been exposed to zinc for 24 weeks and then transferred to control water for 12 weeks revealed that brook trout can translocate or eliminate accumulated zinc. Zinc that continued to accumulate in the kidney of fish in clean water was translocated from other parts of the body (Fig. 2). Benoit et al. (1976) and McKim et al. (1976) observed similar effects in the kidney tissues of brook trout exposed to cadmium and methylmercury, respectively. Joyner (1961) and Chipman et al. (1958) found rapid initial elimination of zinc residues in brown bullheads and pinfish (*Lagodon rhomboides*) followed by a much slower loss of the remaining residues.

Zinc uptake was not rapid in the tissues of adult first-generation brook trout and appeared to be even slower in acclimated second-generation fish. Zinc accumulations in second-generation trout, expressed as micrograms of zinc per gram of tissue or as total micrograms per tissue, tended to be fairly stable between 52 and 93 weeks except for slight increases in liver and kidney tissues of brook trout exposed to 534 μg Zn/liter. This apparent steady state may result from the activation of enzyme systems or by the presence of other homeostatic control mechanisms, which have been suggested to control the tissue levels of zinc in humans (Liebscher and Smith 1968), mice (Cotzias et al. 1962), and fish (Giesy and Wiener 1977).

Muscle tissue from brook trout exposed to as

much as 1,360 μg Zn/liter did not accumulate substantial amounts of zinc. Zinc residues in muscle tissues from this study were close to those observed in several species of Canadian freshwater fish collected from both nonindustrialized and heavily industrialized locations (Uthe and Bligh 1971).

Brook trout gonad tissues did not appear to accumulate significant zinc residues in either first- or second-generation fish. This agrees with data obtained by Matthiessen and Brafield (1977), who found that the concentration factor for gonad tissue of threespine sticklebacks was the lowest of all tissues examined. The lack of zinc in gonads would explain why no significant zinc residues were found in newly spawned eggs at concentrations up to 534 μg Zn/liter. Zinc does accumulate, however, in the embryo or yolk sac, or both, during the embryo stage because residues higher than controls were found in newly hatched larvae exposed to 144, 266, and 534 μg Zn/liter.

The application factor determined for brook trout in this study is between 0.267 and 0.680 based on an MATC between 534 and 1,360 μg Zn/liter and a 96-hour LC50 of 2,000 μg Zn/liter. A complete discussion of other application factors for zinc and other metals and their usefulness is contained in a recent publication by Andrew et al. (in press).

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References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1971. Standard methods for the examination of water and wastewater, 13th edition. American Public Health Association, New York, New York, USA.

Andrew, R. W., D. A. Benoit, J. G. Eaton, J. M. McKim, and C. E. Stephan. In press. Evaluation of an application factor hypothesis. United States Environmental Protection Agency, Ecological Research Series, Duluth, Minnesota, USA.

Bengtsson, B. E. 1974. Effect of zinc on growth of the minnow *Phoxinus phoxinus*. Oikos 25:370–373.

Benoit, D. A. 1974. Artificial laboratory spawning substrates for brook trout (*Salvelinus fontinalis* Mitchill). Transactions of the American Fisheries Society 103:144–145.

Benoit, D. A. 1976. Toxic effects of hexavalent chromium on brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). Water Research 10:497–500.

Benoit, D. A., and G. W. Holcombe. 1978. Toxic effects of zinc on fathead minnows (*Pimephales promelas*) in soft water. Journal of Fish Biology 13: 701–708.

Benoit, D. A., E. N. Leonard, G. M. Christensen, and J. T. Fiandt. 1976. Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). Transactions of the American Fisheries Society 105:550–560.

Benoit, D. A., and F. A. Puglisi. 1973. A simplified flow-splitting chamber and siphon for proportional diluters. Water Research 7:1915–1916.

Brungs, W. A. 1969. Chronic toxicity of zinc to the fathead minnow, *Pimephales promelas*. Transactions of the American Fisheries Society 98:272–279.

Chapman, G. A. 1978. Effects of chronic zinc exposure on sockeye salmon during adult-to-smolt freshwater residency. Transactions of the American Fisheries Society 107:828–836.

Chipman, W. A., T. R. Rice, and T. J. Price. 1958. Uptake and accumulation of radioactive zinc by marine plankton, fish, and shellfish. Unites States Fish and Wildlife Service Fishery Bulletin 58:279–292.

Cotzias, G. C., D. C. Borg, and B. Selleck. 1962. Specificity of zinc pathway through the body: turnover of Zn^{65} in the mouse. American Journal of Physiology 202:359–363.

Crandall, C. A., and C. J. Goodnight. 1962. Effects of sublethal concentrations of several toxicants on growth of the common guppy, *Lebistes reticulatus*. Limnology and Oceanography 7:233–239.

Eaton, J. G. 1973. Chronic toxicity of a copper, cadmium, and zinc mixture to the fathead minnow *Pimephales promelas*. Water Research 7:1723–1736.

Frieden, E. 1972. The chemical elements of life. Scientific American 227:52–60.

Giesy, J. P., Jr., and J. G. Wiener. 1977. Frequency distribution of trace metal concentrations in five freshwater fishes. Transactions of the American Fisheries Society 106:393–403.

Holcombe, G. W., and R. W. Andrew. 1978. The acute toxicity of zinc to rainbow and brook trout: comparisons in hard and soft water. United States Environmental Protection Agency Ecological Research Series, EPA-60013-78-094.

Holcombe, G. W., D. A. Benoit, E. N. Leonard, and

J. M. McKim. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Research Board of Canada 33:1731–1741.

Hurley, D. A., and K. C. Fisher. 1965. The structure and development of the external membrane in young eggs of the brook trout, *Salvelinus fontinalis* (Mitchill). Canadian Journal of Zoology 44:173–190.

Joyner, T. 1961. Exchange of zinc with environmental solutions by the brown bullhead. Transactions of the American Fisheries Society 90:444–448.

Leonard, E. N. 1971. The determination of copper in fish tissues by atomic absorption spectrophotometry. Atomic Absorption Newsletter 10:84–85.

Liebscher, K. L., and H. Smith. 1968. Essential and nonessential trace elements. Archives of Environmental Health 17:881–890.

Matthiessen, P., and A. E. Brafield. 1977. Uptake and loss of dissolved zinc by the stickleback *Gasterosteus aculeatus* L. Journal of Fish Biology 10:399–410.

McKim, J. M., and D. A. Benoit. 1971. Effects of long-term exposure to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Research Board of Canada 28:655–662.

McKim, J. M., and D. A. Benoit. 1974. Duration of toxicity tests for establishing "no effect" concentrations for copper with brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Research Board of Canada 31:449–452.

McKim, J. M., G. F. Olson, G. W. Holcombe, and E. P. Hunt. 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): toxicity, accumulation, distribution, and elimination. Journal of the Fisheries Research Board of Canada 33:2726–2739.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research 1:21–29.

Mount, D. I., and C. E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish—malathion and the butoxyethanol ester of 2,4-D. Transactions of the American Fisheries Society 96:185–193.

Mount, D. I., and R. E. Warner. 1965. A serial-dilution apparatus for continuous delivery of various concentrations of materials in water. United States Public Health Service Publication 999-WP-23, Washington, District of Columbia, USA.

Perkin-Elmer. 1976. Analytical methods for atomic absorption spectrophotometry. September.

Sinley, J. R., J. P. Goettl, Jr., and P. H. Davies. 1974. The effects of zinc on rainbow trout (*Salmo gairdneri*) in hard and soft water. Bulletin of Environmental Contamination and Toxicology 12:193–201.

Spehar, R. L. 1976. Cadmium and zinc toxicity to flagfish, *Jordanella floridae*. Journal of the Fisheries Research Board of Canada 33:1939–1945.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research 3:793–821.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics with special reference to biological sciences. McGraw-Hill, New York, New York, USA.

Uthe, J. F., and E. G. Bligh. 1971. Preliminary survey of heavy metal contamination of Canadian freshwater fish. Journal of the Fisheries Research Board of Canada 28:786–788.

Vladimirov, V. I. 1969. Dependence of the embryonic development and viability of the carp on the trace element zinc. Voprosy Ikhtiologii 9:687–696.